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PHENOLOGICAL BEHAVIORS OF LARGE-LEAVED LINDEN (*TILIA PLATHYPHYLLOS* SCOP.) SEEDLINGS IN DEPENDING ON ENVIRONMENTAL CONDITIONS

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Abstract

The main aim of the present study was to establish the phenological development of seedlings of the widely spread linden species in Bulgaria - Tilia plathyphyllos Scop. in dependence on the environmental conditions. The seeds on 75 and 90 days after flowering were applied for propagation. The beginning and mass appearance following phenological stage as sprouting, cotyledons, first true leaf and third true leaf were recordered. The average daily temperature and sum of rainfall during the vegetation period were calculated. The relations between phenological behaviours and investigated elements of climatic conditions were determinate. The correlations between the average daily temperature on one hand and periods between different stages of seedling development on the other hand were calculated. The regressions analysis between duration of different phenophases and average daily temperature were also done. The significance of the environmental conditions on the phenological development, especially for sprouting and appearing first true leaf was established.

Key words: *propagation, seeds, flowering, temperature, rainfall.*

INTRODUCTION

The linden is one of the widely applied trees for urban, park and alley as well as forest landscaping, especially in the temperate regions of Europe, North America, and Asia. Many scientists have claimed the fact that the linden genus contains many species and indicate that in this genus includes more than 30 species. The main species are *Tilia platyphyllos* Scop. (large-leaved lime), *Tilia cordata* Mill. (small-leaved line) and *Tilia americana* L. (basswood) (Chalupa, 1999). While more basswood is used in America, more large-leaved lime and a small-leaved line has been used in Europe (Farar, 1995). Radoglou et al. (2009) in Europe, there are natural linden forests mainly composed of the following species as *Tilia dasystyla* Stev., *Tilia tomentosa* Moench., *Tilia cordat*a Mill. and *Tilia platyphyllos* Scop.
These natural habitats are seen all over the continent, with the exception of the Scandinavian countries. The species *Tilia tomentosa* Moench. however, is mainly spread linden tree on the Balkan Peninsula
The large-leaved and small-leaved linden are characterized by very good adaptive ability. In these species, this evolutionary development is most lasting and the root system is a stronger development especially at an early age. This is one of the prerequisites for a higher adaptive ability (Shaiyahmentov & Seydafarov, 2013).
De Jaegere et al. (2016) reported that the limiting factor for linden plant development and especially for good formation of the seeds is the temperature. In connection with wider dissemination and successful reproduction, it is necessary to be clarifid the better possibilities for doing so.
The linden spreading in a larger geographical area mainly is determinated by the ecological and climatic factors. In this scope, the main elements are the average January temperatures, the sum of the negative temperatures, the yearly rainfall, the length of the vegetation period, the altitude, the distance to the sea or large water basins (Evarte-Bundere, 2014).
Seydafarov (2012) points out that the water content of the leaves, along with climatic conditions, especially in older trees, is essential for their development. Varaksin and Kladko (2010) have conducted an experiment about the dependences of the three locations on the changes on the phenological development.
The stages of development in linden trees are more prolonged when they are planted nearby

to the areas to highways and with high urban pollution. This prolongation is mostly observed on the seed development and in delay of the ripening. The adaptability is better in large-leaved and small-leaved lime.

In some studies, it is emphasized that in linden seed formation is hampered by lower temperatures. Another opinion expressed Kollas et al. (2011), the temperature at which a significant quantity of viable seeds can be formed may be taken as the lower limit temperature. At such low temperatures, the flowering is changing, pollen formation and spreading, pollen tube growth, fertilization, embryogenesis, and seed maturation are made much more difficult and slower.

The main goal of the present study was to establish the phenological development of seedlings of the widely spread linden species in Bulgaria - *Tilia plathyphyllos* Scop. in dependence on the environmental conditions.

MATERIAL AND METHODS

The experiments were carried out in the Department of Horticulture and in the Department of Botanic and Agrometeorology at the Agricultural University-Plovdiv, Bulgaria in 2015-16 years with seeds of large-leaved lime (*Tilia platyphyllo*s Scop.)

The seeds harvested on 75 and 90 days after the flowering of 35-40-year-old trees in the region of Plovdiv, Bulgaria. Seed collection days are determined, according to the results of our previous experiments (Panchev, 2018). They were sown immediately after harvesting an open-air bed at a distance of 20 cm between the rows and 7cm inside the row.

The experiments were carried out by block method in 4 replicates per 100 seeds for each. The main phenophases of seedling development such as beginning and mass sprouting, beginning and mass cotyledons formation, beginning and mass development of first and third true leaves were established.

The observations were carried out on each sowing seeds and developed seedlings. The presence in 10% of the seeds or seedlings of the above-mentioned was accepted for their beginning, while for mass - it was in 75% (Dimova & Marinkov, 1999).

Based on the data from the daily meteorological observations of Synoptic Station - Plovdiv, located on the Experimental Field of Agricultural University - Plovdiv, the changes of the main agrometeorological factors in interfacial periods are traced. The main factors of the climate as the air temperature and the amount of rainfall (for the phase and 1 day of the period) were recorded. Through them the agro-climatic indicators were determined by inter-phase periods. The mathematical processing of the results was performed on Excel using Visual Basic.

RESULTS AND DISCUSSION

According to Sparks et al. (2000) the oobservations on phenology is very useful, because by means of the obtained results can be assessed the the environmental factors and their impact on plant development. The interaction between temperature and phenological behaviors is very direct and it can serve as a basis for predicting the plant development (Weintraub et al., 2007). The differences between the separate phenological stages of the linden seedlings in the course of one year when using seeds of different ages are relatively small (Table 1). This is probably due to the long period required for seed germination.

A characteristic feature of lime seed is the presence of prolonged dormancy, which also determines the long period from sowing to sprouting. Such opinion is supported by a number of authors. Milev (2007) also finds that lime has a long sprouting period, and for some linden species it can reach up to 18 months. The separate phenophases also pass in short terms. The beginnings of germination are most accelerated in 2016, with harvested seed in 2015, for 75 days seeds it is 201 days after sowing, and for those on 90 days it is 186 days. During all growing seasons, the 90-day seed has been shown to have earlier germination. This may be related to better seed development and more stable build-up of their structures at a later stage of harvest when a more complete botanical maturity occurs (Panayotov, 2015). At the latest seed began to sprout in 2017, 28 and 31 days longer than in the previous year, for seeds on 75 and 90 days after flowering, respectively. During the first year, the differences between the beginning and the period between these two phenophases grows markedly, as between 10 and 20 days were necessary to initiate a mass sprouting phase. In *Tilia plathyphyllos* Scop.average, for the three years of the experiment, the seeds of 75 days after flowering they sprouted for 219.3 days and those of 90 days – for 14 days less.

Much-accelerated development is also observed with regard to the occurrence of the next phase - cotyledons. In many cases, their initial appearance coincides with mass sprouting or the difference is very minimal of about one day. The rate of mass formation of the cotyledons also increased, especially in 2016. This period is higher over the next two years, approximately 3 to 4 days were necessary for the mass development of the cotyledons. It was the shortest in 2016. The effect of environmental conditions is higher on the passage and duration of earlier stage of the plant development (Chmielewski et al., 2004).

In the later stages, the interfacial periods are larger. The earliest, the beginning of the first true leaf was observed in seeds at 75 days - 30.03.2016 or 12 days after germination. For the next two years, it took about 20 days from sprouting to the appearance of the first real sheet. Most days for the mass formation of the first true leaf are counted for the 75-day seed in 2017 and 2018, and for the 90 days in 2018.

The period for the occurrence of third true leaves is even greater. For seeds of the 75th day, it is the longest - 48 days in 2016 after the beginning of the first true leaf and 19 and 15 days in 2017 and 2018, respectively. For these seeds, the mass manifestation of this phase is 2 days after the beginning while in 2017 - 3 days. The period for the seedlings from seeds of 90 days after flowering at the beginning of the third true leaf stage in 2016 is also prolonged - 46 days after the development of the first true leaf. In the next two vegetations, this period is much shorter - 17 and 15 days, in 2017 and 2018, respectively. A similar trend is observed for the mass appearance of this phase. In the first year the period about the mass formation of the third true leaves was significantly higher - 47 days. The development is accelerated in 2017 and 2018 - 19 and 15 days after the mass formation of the first true leaves, but 4 and 3 days after the stage of the beginning of the third true leaves.

Table1. Phenological behaviors of large-leaved lime seedlings

Sowing	2015		2016		2017	
	75 day	90 day	75 day	90 day	75 day	90 day
Indexes	29.08	12.09	17.08	01.09	12.08	27.08
	2016		2017		2018	
Beginning of sprouting	18.03	17.03	4.04	5.04	28.03	27.03
Mass of sprouting	19.03	18.03	15.04	17.04	09.04	11.04
Beginning of cotyledons	19.03	18.03	15.04	17.04	09.04	11.04
Mass of cotyledons	21.03	19.03	18.04	20.04	13.04	13.04
Beginning of first true leaf	30.03	31.03	24.04	23.04	16.04	15.04
Mass of first true leaf	1.04	1.04	27.04	25.04	19.04	18.04
Beginning of third true leaf	17.05	16.05	13.05	10.05	01.05	30.04
Mass of third true leaf	19.05	17.05	16.05	14.05	03.05	03.05

Information on phenological characteristics and biological requirements, especially of the temperature, can help to define better the area with optimal conditions for development of the given species (Florea & Stefănescu, 2009). In general, the meteorological conditions exert an influence on the rate of linden seedling development, but their manifestation is different for the two investigated terms of the seeds. Their impact on the first variant - 75 days is more significant.

At the seeds on 75 days on the rate of development during sowing to mass sprouting exert an influence on the air temperature as well as precipitation distribution for one day period (mm/day) (Figure 1 and Figure 2). In this case, the temperature of the air is determined. Increasing the average air temperature from 4 to 12°C reduces the period from sowing to mass sprouting by almost half (Table 2). During the period of mass sprouting to the mass formation of cotyledons the average air temperature is also decisive (Figure 3). From the mass formation of cotyledons to the mass appearance of the first true leaves, both the air temperature and the amount of precipitated rainfall have an influence on the rate of development (Figure 4 and Figure 5). The increase of the air temperature from 2 to 16° C decreases the period three times (Table 3). The amount of rainfall has a significant effect on the rate of development during the last period, of mass formation of the first true leaves to the mass formation of third true leaves (Figure 6). The rate of development is the fastest in the amount of precipitation 10-20 mm. The increase of the precipitation from 10 to 90-100 mm significantly slows down the rate of development and prolongs the period to 48-53 days (Table 4).

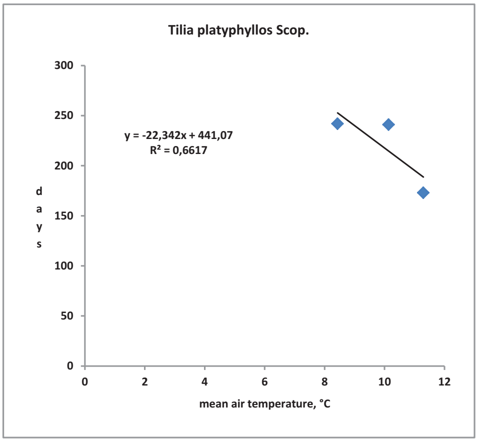

Figure 1. Dependence between the duration of the sowing period and the sprouting of 75-days linden seeds and the average air temperature.

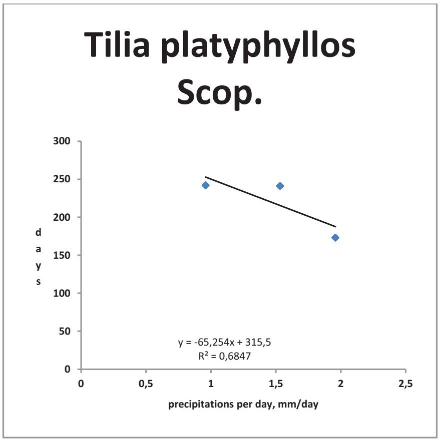

Figure 2. Dependence between the duration of the sowing to sprouting of 75-days linden seeds and the amount of rainfall for one day of the per period.

Table 2. Influence of the air temperature on the rate of the linden development during the period seed-to-sprouting for 75-day seed

t, °C	4	6	8	10	12
N, days	351	307	262	218	173

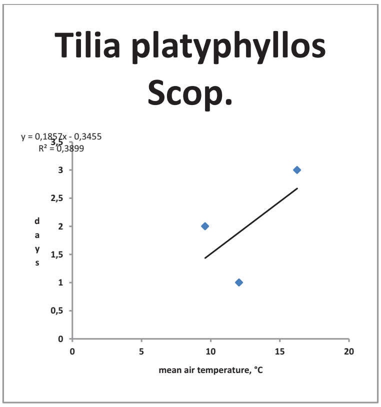

Figure 3. Dependence between the duration of the period of mass sprouting to mass development of the cotyledons in seedlings of 75-days linden seeds and the average air temperature for this period.

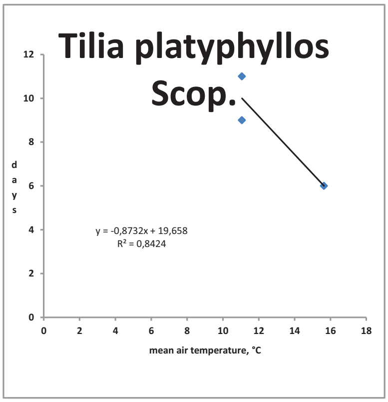

Figure 4. Dependence between the duration of the period of mass development of the cotyledons to mass development of first true leaf in seedlings of 75-days linden seeds and the average air temperature for this period.

Table 3. Influence of the air temperature on the rate of the development during the period of mass formation of the cotyledons to mass formation of first true leaf in linden seedlings from 75-days seeds.

t, °C	2	4	6	8	10	12	14	16	18	20
N, days	18	16	14	13	11	9	7	6	4	2

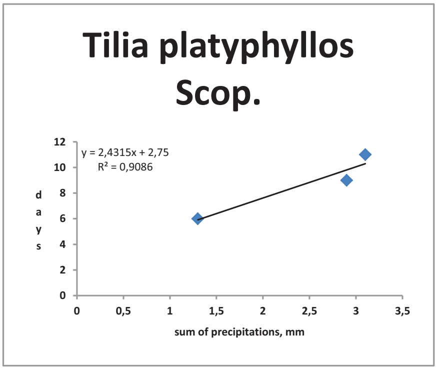

Figure 5. Dependence between the duration of the period of mass development of the cotyledons to mass formation of first true leaf in seedlings of 75-days linden seeds and amount of rainfall for this period.

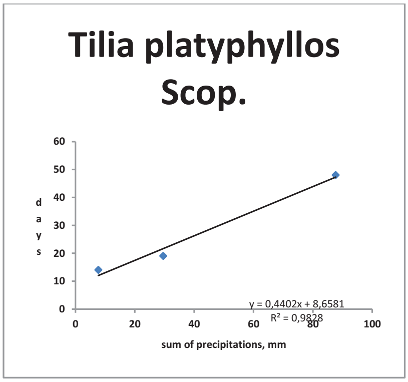

Figure 6. Dependence between the duration of the period of mass development of first true leaf to mass formation of third leaf in seedlings of 75-days linden seeds and amount of rainfall for this period.

Table 4. Influence of the rainfall on the rate of the development during the period of mass formation of the first true leaf to mass formation of third leaf in linden seedlings from 75-days seeds

ΣR, mm	10	20	30	40	50	60	70	80	90	100
N, days	13	17	22	26	31	35	39	44	48	53

In the variant with 90-days seeds, the impact of meteorological conditions on the rate of development of linden seedling during the first two phases, from sowing to mass sprouting and then to mass cotyledon formation is insignificant.

During the period of mass formation of cotyledons to a mass appearance of the first true leaf, the influence of rainfall is decisive and with them the close correlation and regression dependence were established (Figure 7).

During the period of mass formation of the first true leaf to the mass formation of a third true leaf, the high influence was observed about the effect of the air temperature and the amount of precipitations and close correlation and regression dependencies were established with them (Figure 8 and Figure 9).

Increasing the air temperature by 5°C (from 14 to 19° C) decreased this period 10 times, therefore every degree of temperature increase reduces the duration of the period by an average of 4-5 days (Table 5).

Increasing precipitation from 10 to 100 mm extends the period from first to third true leaf more than three times, i.e. every 10 mm increase in rainfall prolongs the period by an average of 3-4 days (Table 6).

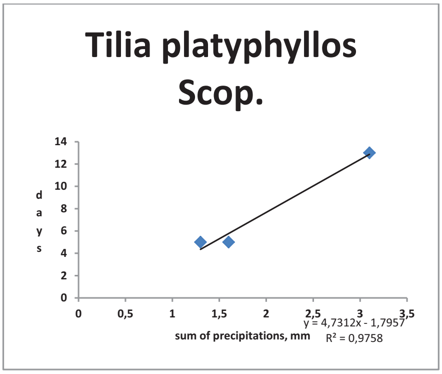

Figure 7. Dependence between the duration of the period of mass development of the cotyledons to mass formation of first true leaf in seedlings of 90-days linden seeds and amount of rainfall for this period.

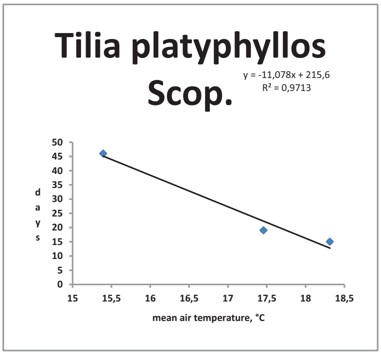

Figure 8. Dependence between the duration of the period of mass formation of first true leaf to mass development of third leaf in seedlings of 90-days linden seeds and average air temperature for this period.

Table 5. Influence of the air temperature on the rate of the development during the period of mass formation of the first true leaf to mass formation of third leaf in linden seedlings from 90 days seeds.

t, °C	14	15	16	17	18	19
N, days	49	49	38	27	16	5

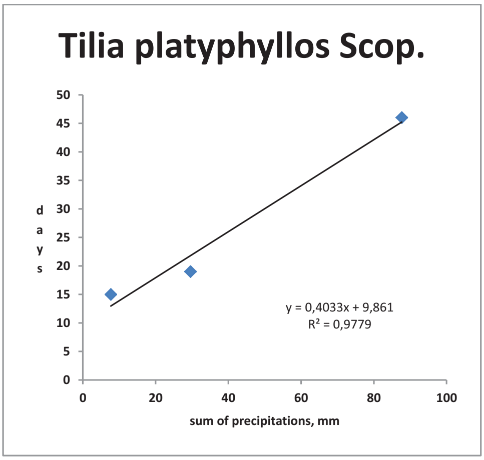

Figure 9. Dependence between the duration of the period of mass formation of first true leaf to mass development of third leaf in seedlings of 90-days linden seeds and amount of rainfall for this period.

Table 6. Influence of the rainfall on the rate of the development during the period of mass formation of the first true leaf to mass formation of third leaf in linden seedlings from 90-days seeds.

ΣR, mm	10	20	30	40	50	60	70	80	90	100
N, days	14	18	22	26	30	34	38	42	46	50

CONCLUSIONS

The passage of the initial phenophases of the seedlings large-leaved linden (*Tilia plathyphyllos* Scop.) is in shorter terms. The phenophases beginning and mass formation of the first and third true leaves are characterized by a longer period of the development.

More accelerate development was observed in seedlings grown from seeds at 90 days after flowering in comparison to those of day 75.

The most accelerate is sprouting of seed in 2016. The seeds of 75-day after flowering from large-leaved linden sprouted average for 219.3 days and those of 90 days - for 14 days less. Significantly short is the period of development of phenophase cotyledons.

Relatively longer is the period for the beginning of the formation of the first true leaf, but the difference with the mass manifestation of this phase is insignificant.

The period for the formation of a third true leaf is the longest, approximately 45 days after the start of the sprouting.

Between both studied terms for the harvesting of linden seeds the differences to their response to environmental factors were identified. The seeds of 75 days after flowering are more sensitive to changes in weather conditions that have a strong impact on the development rate of large-leaved linden throughout the whole period – from sowing to mass third leaf formation.

The seedlings from 90-days seed are characterized by a slower growth rate in less rainfall, and a faster rate in a higher amount of rainfall than these of 75-day seeds.

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